The Effects of Nicotinamide on Heat Resistance of Spores of *Bacillus cereus* T

Heat resistance is one of the most important characteristics of the bacterial endospore. Since the discovery of dipicolinic acid (DPA) in the bacterial endospores[1], evidence has been steadily accumulated to implicate DPA in the heat resistance of the bacterial endospores. The protection of some enzymes and proteins by DPA against heat denaturation[2-4] has also been demonstrated. Indirect evidence has also been adduced through the production of DPA deficient spores sensitive to heat by using inbalanced media[5], specific inhibitors[6] and DPA less mutants[7]. In the latter two cases the effects could be reversed by exogenous DPA.

Recently UPRETI et al.[8] have shown that picolinamide specifically inhibits DPA synthesis leading to the production of DPA deficient heat sensitive spores. The effect of picolinamide cannot be reversed by zinc. DPA was shown to activate the soluble reduced diphosphopyridine nucleotide (DPNH) oxidase of spores by electron accepting mechanism[9] and also to protect it against heat inactivation[2]. It was thought that picolinamide may be interferring with the role of DPA as an electron acceptor in the spore. Therefore a study of the effects of nicotinamide was undertaken.

Materials and methods. *Bacillus cereus* strain T was used throughout these investigations. The active culture technique was used and the organism was allowed to grow and sporulate in a glucose yeast extract-salts medium (G medium). Total viable counts (TVC) were made by plating suitable dilutions on nutrient agar. Octyl alcohol kills vegetative cells and germinated spores of this organism. Octyl alcohol stable counts (OSC) were made by using octyl alcohol saturated water for dilution in making plate counts. Heat stable counts (HSC) were made by plating suitable dilutions, after first heating the suspension at 80 °C for 30 min. The OSC gives the total number of spores while the HSC gives the number of heat resistant spores in the culture.

Results and discussion. The effects of nicotinamide and nicotinic acid on heat stability are given in Table I. The effects of exogenous DPA on nicotinamide induced heat sensitivity are given in Table II. The results show that whereas nicotinic acid has no effect, nicotinamide

Table I. Effect of nicotinamide and nicotinic acid on heat stability of spores of *Bacillus cereus* T

Additions to G medium	After 30 h incubation[a]			
	TVC (ml)	OSC (ml)	HSC (ml)	HSC (%)
None (Control)	2.0×10^8	2.1×10^8	2.1×10^8	100
Nicotinamide (2 mg/ml)	2.1×10^8	2.2×10^8	6.0×10^5	0.27
Nicotinic acid (2 mg/ml)	1.0×10^8	1.0×10^8	1.0×10^8	100

[a] Incubated on a rotary shaker at 30 °C (± 1 °C).

Table II. Effect of DPA on the production of heat sensitive spores of *Bacillus cereus* T in the presence of nicotinamide

Addition to G medium	After 30 h incubation		
	OSC (ml)	HSC (ml)	HSC (%)
None (Control)	2.1×10^8	2.2×10^8	100
Nicotinamide (2 mg/ml)	1.9×10^8	5.0×10^5	0.26
DPA (0.5 mg/ml)	2.0×10^8	2.2×10^8	100
Nicotinamide (2 mg/ml) + DPA (0.5 mg/ml)	2.0×10^8	5.5×10^5	0.27

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specifically inhibits the synthesis of DPA and the development of heat resistance. However, exogenous DPA cannot reverse the nicotinamide induced heat sensitivity. Further, spores produced in the presence of nicotinamide contained as much as 40% of the normal DPA as determined by the method of JANSSEN et al.[11]. It has been shown that if the DPA content of the spore is above 1.2%, viability is independent of the DPA content, and that, as the level of DPA is reduced below 1.2%, the proportion of non-viable spore-like bodies increased.

In the literature there has been no reference so far to the role of DPA as an electron acceptor in the heat resistance of bacterial endospores. The results obtained in our laboratory with picolinamide and nicotinamide suggest that in the heat resistance of bacterial endospores, the role of DPA as an electron acceptor may be important[12,14].

Zusammenfassung. Nicotinamide hemmt die Synthese von Dipicolinsäure (DPA) und die Entwicklung der Hitzeresistenz von *Bacillus cereus* T Sporen. Nicotinsäure hat keinen Effekt. Zugabe von DPA kann die Hitzsensitivität, die durch Nicotinamide induziert wurde, nicht rückgängig machen.

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